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ORIGINAL CONTRIBUTION

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Bioactive peptides and proteins from foods: indication for health effects

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Abstract Some dietary proteins cause specific effects going beyond nutrient supply. A number of proteins seem to act directly in the intestine, such as IGFs, lactoferrin and immunoglobulins. Many substances, however, are peptides encrypted in intact molecules and are released from their encrypted position by enzymes during gastrointestinal transit or by fermentation or ripening during food processing. Among food-derived bioactive proteins and peptides from plants and animals, those obtained from milk are known in particular. Numerous effects have been described after in vitro and animal trials for bioactive proteins and peptides, such as immunomodulating, antihypertensive, osteoprotective, antilipemic, opiate, antioxidative and antimicrobial. This article reviews the current knowledge of the existence of bioactive proteins and of in vitro bioactivity and the present evidence of health effects exerted by such substances or products containing bioactive compounds. For example, there is evidence for the antihypertensive effects of milk products fermented with *Lactobacillus helveticus* containing the tripeptides IPP and VPP, which inhibit angiotensin converting enzyme, and for osteoprotective effects by milk basic protein. There is less profound evidence on the immunomodulating effects of lactoferrin and postprandial triglyceride reduction by a hydrolysate of bovine hemoglobin.

Key words bioactive proteins – bioactive peptides – food proteins – immunomodulation – antihypertensive activity – osteoprotection – antilipemic activity

Introduction

In recent years extensive scientific evidence has been provided for the existence of biological active peptides and proteins derived from foods that might have beneficial effects upon human health. The aim of this article is to review the current evidence for the health benefits of bioactive proteins and peptides obtained from foods for human health, with emphasis on immunomodulating, antihypertensive, osteoprotective and antilipemic substances.

Definition

According to a previously suggested definition, "bioactive substances" comprise "food components

that can affect biological processes or substrates and, hence, have an impact on body function or condition and ultimately health" [84].

Since dietary components are likely to have an impact on biological processes if they are consumed in large enough quantities, this definition is usually refined by two caveats:

1. To be considered "bioactive", a dietary component should impart a measurable biological effect at a physiologically realistic level;
2. The "bioactivity" measured has to have the potential (at least) to affect health in a beneficial way, thus excluding from this definition potentially damaging effects (such as toxicity, allergenicity and mutagenicity, which are undoubtedly a reflection of "bioactivity" in its broadest sense) [84].

Besides intact proteins such as growth factors, immunoglobulins and lactoferrin, increasing interest is focused on peptides which may be released from proteins by enzymatic activity during intestinal digestion or by fermentation processes during food processing or ripening.

Sources of bioactive proteins and peptides

According to present knowledge, bovine milk, cheese and dairy products seem to be by far the greatest sources of bioactive proteins and peptides derived from food. This may be due to the particular purposes to which milk is dedicated beyond nutrition in the first months of life: "*Bioactive substances in milk and colostrum is mother language on a substrate basis*" [84]. Bioactive peptides and proteins, however, are gained from other animal and plant sources, too. In bovine blood, gelatin, meat, eggs and various fish species like tuna, sardine, herring and salmon as well as in wheat, maize, soy, rice, mushrooms, pumpkin and sorghum, bioactive proteins have been directly detected or detected after release by hydrolysation or fermentation.

Release of encrypted peptides

Bioactive peptides may be encrypted in the amino acid sequence of a larger protein. These peptides usually consist of 3–20 amino acids and are released from the original protein after degradation.

There are three ways that encrypted peptides can be liberated:

1. In vivo during digestion by digestive enzymes like trypsin
2. In vivo during digestion by microbial enzymes

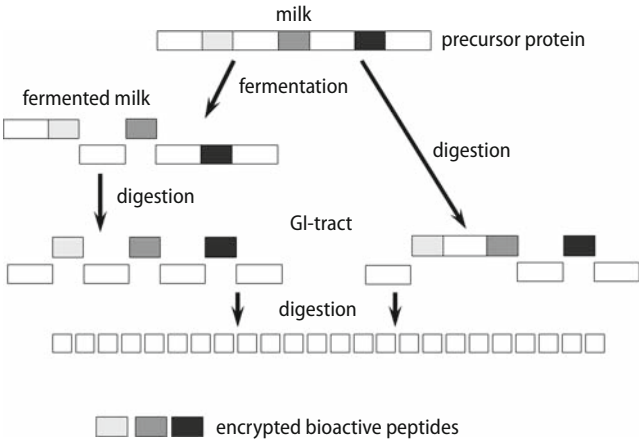

Fig. 1 Scheme of possible differences between peptides released from precursor proteins by fermentation and/or gastrointestinal digestion

3. In vitro during food processing or ripening by isolated or microbial enzymes (e.g. by *Lactobacillus helveticus*).

A number of bioactive peptides were shown to be released by trypsin, e.g. FFVAPFPEVFGK and TTMPLW from α_{s1}-casein and AVPYPQR from β-casein [107]. Bioactive peptides may be liberated from food proteins throughout the whole intestine, and they can display bioactivity in the small and large bowel. However, since most food proteins are degraded during their transit through the small intestine and since microbial activity predominantly occurs in the large intestine, the release by microbial enzymes during digestion is conferred to proteins reaching this site of the intestine. Indeed, some proteins like lactoferrin or immunoglobulins have been shown to partly escape degradation in the small intestine [18, 72]. Plasmin from milk or enzymes from bacterial starter cultures may also degrade proteins. In comparison to digestive enzymes, microbial enzymes, either in the gut or in the food, use different cleavage sites. Thus, peptides liberated by these enzymes may differ from those liberated by digestive enzymes. They may also be precursors for peptides released in the intestinal tract (Fig. 1). Accordingly, Yamamoto [107] did not find an antihypertensive effect of a tryptic digest of casein. By the digestion with extracellular proteinase from *L. helveticus*, KVLPVPQ was released which exerted a weak antihypertensive action (IC_{50} > 1,000 μM). The following pancreatic digestion resulted in a shorter peptide (KVLPVP) with a much higher potential for ACE inhibition (IC_{50}: 5 μM) [107].

Site of action

It is well known that di- and tripeptides are easily absorbed in the intestine [1, 26]. Little, however, is known about the absorption of bioactive peptides with larger

molecular weight in the intestine. In order to exert antihypertensive effects, for example, peptides have to be absorbed from the intestine and reach the target cells in the blood vessels in substantial concentrations. Indeed, the administration of Calpis™, a milk product fermented by *L. helveticus* and *Saccharomyces cerevisiae* and containing the tripeptides VPP and IPP, to rats resulted in a lowering of blood pressure. Six hours after the administration of Calpis™, VPP and IPP could be detected by HPLC in aortal tissues and angiotensin I-converting enzyme(ACE)-activity in the aorta was lower compared to the control group which was given saline [47]. The ACE-inhibitory peptide VPP has been reported to pass the membrane of CaCo-2 cell monolayers in a significant amount, and paracellular diffusion has been proposed as the transport mechanism [75].

Most known bioactive peptides are not absorbed in the intestinal tract. Hence, many proteins and peptides may either act directly in the intestinal tract or via receptors and cell signalling in the gut.

Some dietary proteins may exhibit specific effects going beyond nutrient supply. A number of proteins seem to act directly in the intestine, such as IGFs, lactoferrin and immunoglobulins. Many substances, however, are peptides encrypted in intact molecules and are released from their encrypted position by enzymes during gastrointestinal transit or by fermentation or ripening during food processing. Among food-derived bioactive proteins and peptides from plants and animals, those obtained from milk are known in particular. Numerous effects have been described after in vitro and animal trials for bioactive proteins and peptides, such as immunomodulating, antihypertensive, osteoprotective, antilipemic, opiate, antioxidative and antimicrobial.

Effects

Numerous bioactivities have been described for peptides released from dietary proteins by enzymatic proteolysis including opiate, antithrombotic, antihypertensive, immunomodulating, antilipemic, osteoprotective, antioxidative, antimicrobial, ileum contracting, anticariogenic and growth promoting properties. Specific peptides can have one or more different biological effects. In this review immunomodulating, antihypertensive, osteoprotective and antilipemic proteins and peptides are described in more detail, whereas other effects are described briefly.

Immunomodulatory proteins and peptides

Most of the studies on the effects of proteins and peptides on immunomodulation have been conducted in vitro with cells of the specific and unspecific immune system, fewer studies exist on the immune function in animals and humans.

Several milk proteins have been reported to modulate lymphocyte proliferation in vitro such as whole casein [61], α-, β-, κ-casein [9, 61, 106], whole whey protein [5], lactoferrin [66], lactoperoxidase [105], milk growth factors [65] and milk immunoglobulin G [41]. These findings show that milk feeding facilitates passive immunity in neonates and, thus, contributes to their protection against harmful environmental pathogens [50]. It is important to note that milk-derived proteins and peptides have immunoregulatory features. Pure stimulation of the newborn's immune system alone would lead to an overreaction of the immune response and undesired effects, such as chronic inflammation [13]. Some milk factors like lactoferrin and lactoperoxidase exhibit their immunomodulating actions only in their isolated form. Their effects are diminished if they are combined with other milk factors [14, 105]. This suggests that the immunomodulatory action of primary milk proteins is well balanced and may get out of control after isolation or neutralisation of certain components [13].

Lactoferrin, an iron binding glycoprotein which occurs in the milk of all mammals, shows diverse effects on the host defence system. Apart from its function as a growth factor and its antimicrobial action, lactoferrin was found to exhibit various immunomodulating effects. For example, lactoferrin and lactoferrin-derived peptides have been reported to influence cytokine production in cell culture experiments which are involved in immune and inflammatory actions of the body [14]. Roos et al. [71] investigated supplementation with 0.9 g lactoferrin on immunoresponse in 95 elderly subjects before and after influenza vaccination in a double blind placebo controlled study. On day 0, i.e. before vaccination, the percentage of granulocytes of the total leukocytes was significantly higher in the lactoferrin group than in the placebo group. A similar effect was found for the monocytes 7 days after vaccination. The phagocytosis activity of the granulocytes was significantly enhanced in the lactoferrin group 1 day after vaccination.

Glycomacropeptides (GMPs) from κ-casein have been shown to possess inhibitory properties in vitro on the proliferation of mouse splenocytes [61]. Thereby carbohydrate-rich GMPs exhibit a stronger immunosuppressive effect than GMPs with a low sugar content [60].

Two chemically synthesized peptides, YG and YGG, which correspond to fragments of bovine κ-casein and α-lactalbumin, exert a stimulating effect on the proliferation of human peripheral blood lymphocytes in vitro [38]. In these studies, β-casomorphin-7 and β-casokinin-10 suppressed lymphocyte

proliferation in low concentrations and enhanced it in higher concentrations.

α_{s2}- and β-Casein, digested with pancreatin and trypsin, induced a significant inhibition of the proliferation of in vitro cultured murine spleen lymphocytes and Peyer's patch cells. However, the same proteins, enzymatically hydrolyzed with pepsin and chymotrypsin, did not show any effect on mitogen stimulated cells [60].

The phosphoserine-rich residues 59–79 from bovine α_{s1}-casein, generated by bovine trypsin digestion, demonstrate a mitogenic activity and a stimulatory activity on immunoglobulin production in mouse spleen cells. The phosphoserine-rich residues 1–25 from β-casein have been shown to possess similar properties on the proliferation of mouse spleen cells [27].

Casein, hydrolysated by *Lactobacillus GG* and digestive enzymes (pepsin and trypsin), has been reported to yield compounds possessing both stimulating and suppressing effects on lymphocyte proliferation [91].

A proline-rich polypeptide (PRP) isolated from ovine colostrum is known for its immunoregulatory properties. A synthetic nonapeptide, which correspond to a peptide isolated from a chymotrypsin digest of PRP, and its C-terminal penta- and hexapeptides have been found to stimulate splenic antibody response to foreign erythrocyte antigens in mice when administered 3 h before immunization [30]. In further investigations, evidence has been provided to demonstrate that PRP activates cytokine production by murine macrophages and induces growth and differentiation of resting B-lymphocytes [34].

Tuftsin, a tetrapeptide (TKPR), is generated by the digestion of heavy chain F_c region of bovine and human immunoglobulin G with endopeptidase and leukokininase [54]. Werner et al. [104] reviewed the evidence on immunomodulatory effects of human tuftsin, including leucocyte chemotaxis and phagocyte motility, enhancement of phagocyte oxidative metabolism and antigen processing, and increase in monocyte- and NK cell-mediated tumor cell cytotoxicity.

Marnila and co-workers [46] investigated the impact of the response of specific antibodies to helicobacter, enriched from the colostrum of hyperimmunized cows, on *Helicobacter felis*-infected Balb/c mice. The preparation reduced the colonization of gastric antrum by *H. felis* in comparison to a control group.

A commercial fish protein hydrolysate has been found to increase the number of IgA+ cells as well as IL-4, IL-6 and IL-10 in the lamina propria of the small intestine in mice [19]. In Table 1 the proposed mechanisms of some immunomodulatory proteins and peptides are summarized.

Antihypertensive proteins and peptides

Angiotensin I-converting enzyme (ACE; peptidyldipeptide hydrolase, EC 3.4.15.1) plays a major role in the regulation of blood pressure. Within the renin–angiotensin system, ACE catalyses the conversion from angiotensin I to angiotensin II, a hormone which results in vasoconstriction, and subsequently in an increase of blood pressure. In addition, ACE degrades bradykinin which has vasodilatatory properties. Thus,

Table 1 Immunomodulatory proteins and peptides

Protein/peptide	Effect	Model	Refs
Caseins (and digests)	T-lymphocyte proliferation ↑	Cell culture	[9, 27, 38, 106]
	T-lymphocyte proliferation ↓	Cell culture	[61, 38]
	Immunoglobulin secretion ↑	Cell culture	[27]
Whey	Lymphocyte blastogenesis ↓	Cell culture	[5]
GMP	Splenocyte proliferation ↓	Cell culture	[61]
YG/YGG	Lymphocyte proliferation ↑	Cell culture	[38]
Milk Ig G	Antibody secretion ↓	Cell culture	[65]
Lactoperoxidase	T-cell mitogenesis ↓	Cell culture	[105]
Lactoferrin	Cytokine release ↓	Cell culture	[14]
	Mammary gland mononuclear cell proliferation ↑	Cell culture	[66]
	Granulocyte phagocytosis ↑	Human	[71]
Prolin-rich polypeptides (and derivates)	B-lymphocyte growth, differentiation ↑	Cell culture	[34]
	Antibody secretion ↑	Animal	[30]
Tuftsin	Leucocyte chemotaxis ↑	Cell culture	[104]
	Phagocyte motility, oxidative metabolism ↑		
	Monocyte, NK-cell cytotoxicity ↑		
Colostrum preparation	*H. felis* colonisation ↓	Animal	[46]
Fish protein	IgA-, IL-4-, IL-6-, IL-10-positive cells ↑	Animal	[19]

GMP glycomacropeptide, *NK-cell* natural killer cell
↑/↓/ → : Increase/decrease/no effect

ACE-inhibitiors lower hypertension and are believed to prevent cardiovascular diseases.

The group of Yamamoto [108] and Maeno [44] obtained an antihypertensive effect with α_{s1}- and β-casein hydrolysates by using extracellular lactobacillus proteases. VPP and IPP were identified as antihypertensive peptides yielded by fermenting milk proteins with *L. helveticus* and *Saccharomyces cerevisiae* [55]. A sour milk product fermented by these germs and containing these peptides (Calpis™) reduced arterial blood pressure in rats [47] and humans [28]. Accordingly, Seppo et al. [85] and Jauhiainen [31] found a reduction in the blood pressure of hypertensive subjects after a 21-week-and a 10-week-administration of 150 ml/day sour milk products which were fermented with *L. helveticus* and contained the same bioactive peptides, compared to a control group. There were no adverse effects detectable for these tripeptides after treatment [15].

β-Lactoglobulin and α-lactalbumin release several ACE-inhibitory peptides after digestion with proteolytic enzymes [52, 64]. ACE-inhibitory peptides have also been isolated from hydrolysates of gelatin [59], γ-zein from corn [51, 109], wheat germ [48], hordein from barley [23], ovalbumin [110], dried bonito [22], sunflower [49] and garlic [90]. Kanauchi et al. [37] showed an ACE-inhibitory effect in vitro and a blood pressure lowering effect in spontaneous hypertensive rats by a peptide fraction from brewer's yeast.

Endothelin is an even stronger vasoconstrictor than angiotensin II. The membrane bound endothelin-converting enzyme (ECE, E.C. 3.4.24.71) converts the precursor endothelin to the active endothelin. Thus, an inhibition of ECE leads to lower blood pressure.

Okitsu et al. [58] investigated the endothelin-converting enzyme-inhibitory properties of pepsin digests of bonito pyrolic appendix and beef and found 40 and 45% enzyme inhibition, respectively. Maes et al. [45] used endothelin-1 (ET-1), released from endothelial cells, to assess antihypertensive peptide activity in a more global approach to the complex vasoregulation. The whey-derived peptide ALPMHIR, a product of a tryptic β-lactoglobin digest, was found to inhibit the ET-1 release from endothelial cells by 29% in a 1 mM concentration compared to captopril (0.1 mM), a well known inhibitor of ET-1, which led to a 42% inhibition of ET-1 release. Antihypertensive actions of proteins and peptides are summarized in Table 2.

Table 2 Antihypertensive actions of some proteins and peptides

Protein/peptide	Effect	Model	Refs
α_{s1}- and β-casein	ACE ↓	In vitro	[108, 44]
	Hypertension ↓	Animal	
VPP/IPP	ACE ↓	In vitro	[55]
	Blood pressure ↓	Animal	[47]
		Human	[28, 31, 85]
Peptides from			
β-Lactoglobulin	ACE ↓	In vitro	[52, 64]
α-Lactalbumin	ACE ↓	In vitro	[52, 64]
Gelatin	ACE ↓	In vitro	[59]
γ-Zein	ACE ↓	In vitro	[51, 109]
Wheat germ	ACE ↓	In vitro	[48]
Hordein	ACE ↓	In vitro	[23]
Ovalbumin	ACE ↓	In vitro	[110]
Sunflower	ACE ↓	In vitro	[49]
Garlic	ACE ↓	In vitro	[90]
Bonito	ACE ↓, ECE ↓	In vitro	[22, 58]
Beef	ECE ↓	In vitro	[58]
Whey	ET-1 release ↓	Cell culture	[45]
Brewer's yeast	ACE ↓	In vitro	[37]
	Blood pressure ↓	Animal	

ACE angiotensin I-converting enzyme, *ECE* endothelin-converting enzyme, *ET-1* endothelin 1, ↑/↓/ → increase/decrease/no effect

Osteoprotective proteins and peptides

A higher amount of soluble or available calcium in the intestine contributes to improve bone mineralisation and by this to prevent the chronic disease osteoporosis. Beside calcium, milk is considered to contain other components effective for bone health. The osteoprotective features of casein and casein-derived peptides have been known for more than 50 years and were reviewed by Scholz-Ahrens and Schrezenmeir [82]. Whole casein, when compared to other proteins like gelatine, gluten and dephosphorylated casein [77], whey protein or soy protein isolate [39, 53], increased the absorption of intestinal calcium in rats. In some cases, no effects were observed on calcium retention and bone mineral content [40]. Casein fed rats showed a significantly higher calcium retention compared to rats fed on whey protein in the presence of phytate for 24 weeks [79]. This effect was obvious only in aged adult rats.

Colloidal calcium is easily absorbed in the small intestine. Due to their ability to form soluble complexes with Ca^{2+} phosphate, casein phosphopeptides (CPPs) can enhance intestinal calcium absorption. Sato et al. [76] reported that CPP isolated from bovine β-casein accelerated the absorption of instilled $^{45}CaCl_2$ from the intestine and stimulated femur calcification. Scholz-Ahrens et al. [80–82] showed in long term experiments over 12 weeks with minipigs, a model that is closer to humans than the rat, only small effects on calcium absorption and bone metabolism by feeding casein, compared to whey protein. Effects on calcium absorption and parameters of calcium and bone metabolism could only be observed at specific experimental conditions, i.e. at a lower content of dietary calcium or during vitamin D deficiency. In the presence of low dietary calcium soluble calcium in the

intestine and calcium retention were lower and plasma concentration of calcium and parathyroid hormone (PTH) higher in minipigs fed casein compared to whey protein. In the presence of a diet with a high calcium content but free of vitamin D bone mineral density and plasma concentration of $25(OH)D_3$ was higher and $1,25(OH)_2D_3$ lower in animals fed casein compared to whey protein [82]. Narva et al. [57] investigated the influence of CPP-enriched milk on acute 24-h-calcium metabolism in postmenopausal women. They did not find any effect on intact PTH, ionized calcium, total calcium, phosphate and urinary calcium excretion.

Also, whey components have shown positive effects upon bone metabolism. Cell culture investigations as well as animal and human trials with milk whey protein, especially its basic protein fraction (milk basic protein, MBP), indicated that MBP promotes bone formation and suppresses bone resorption. MBP was found to suppress the formation of pits by isolated osteoclasts from rabbit dose-dependently [96]. Ovariectomized rats showed a significantly higher bone mineral density (BMD), a higher breaking energy of the excised femur, and lower urinary levels of deoxypyridinoline when fed with 0.01 or 0.1% MBP, in comparison to a group fed with a control diet [96].

In a human study with healthy adult women, a daily supplementation of 40 mg MBP over 6 months resulted in a significantly higher BMD in the left calcaneus and a significantly lower urinary cross-linked *N*-teleopeptides of type-I collagen/creatinine and deoxypyridinoline/creatinine excretion in comparison to a placebo group [2].

In a 16-day-trial with healthy adult men a daily supplementation of 300 mg MBP resulted in a significant increase in the serum osteocalcin concentration and a significant decrease in urinary cross-linked *N*-teleopeptides of type-I collagen excretion [101], indicating that bone formation was stimulated while bone resorption was reduced.

Lactoferrin is a more recently described factor with a beneficial potential for bone and an anabolic factor in osteoporosis [12, 56].

Reviewing the reports on the osteoprotective effects of food-derived proteins and peptides, it becomes clear that evidence for the benefit of these substances on bone formation should be based on long-term studies. Both the casein and the whey components of milk seem to possess remarkable beneficial potential for Ca absorption and bone metabolism. Whether this potential becomes relevant obviously depends on age and on the calcium- and vitamin D-content of the diet. Moreover, it depends on the background diet. A positive effect on calcium absorption by CPPs was observed in human subjects when the diet was based on rice but not on whole grain [25]. Table 3 gives an overview of osteoprotective proteins and peptides.

Table 3 Osteoprotective proteins and peptides

Protein/peptide	Effect	Model	Refs
Casein	Absorption of intestinal calcium ↑	Animal	[77, 53, 39]
	Calcium retention ↑	Animal	[79]
	Calcium retention →	Animal	[40]
	Bone mineral content →		
at low dietary calcium	Soluble calcium in the intestine ↓	Animal	[82]
	Calcium retention ↓		
	Plasma calcium ↑		
	PTH ↑		
at high dietary calcium and no vitamin D	Bone mineral density ↑	Animal	[82]
	$25(OH)D_3$ ↑		
	$1,25(OH)_2D_3$ ↓		
CCPs	Calcium absorption ↑	Animal	[76]
	Femur calcification ↑		
depending on diet	Calcium absorption ↑/ →	Human	[25]
	Calcium metabolism →	Human	[57]
Milk basic protein	Formation of pits ↓	Cell culture	[96]
	Bone mineral density ↑	Animal	[96]
	Bone breake force resistance ↑		
	Urinary deoxypyridinoline ↓	Human	[2]
	Bone mineral density ↑		
	Urinary marker of cross-links ↓	Human	[101]
	Osteocalcin ↑		
	Urinary marker of cross-links ↓		
Lactoferrin	Anabolic factor in osteoporosis	Cell culture, animal	[12, 56]

PTH parathyroid hormone, *CCPs* casein phosphopeptides, *$25(OH)D_3$* 25-Hydroxyvitamin D_3, *$1,25(OH)_2D_3$* 1,25-Dihydroxyvitamin D_3
↑/↓/ → : increase/decrease/no effect

Antilipemic proteins and peptides

The enzyme triacylglycerol lipase (E.C. 3.1.1.3) and its protein cofactor colipase are essential for the cleavage of dietary triglycerides and the subsequent absorption of fatty acids in the intestinal tract. A reduced lipase activity in the gut can result in an inhibition and/or a delay of fat assimilation and, consequently, in a decrease of postprandial triglyceride levels in the blood. High postprandial triglyceride levels are associated with insulin resistance, precocious atherosclerosis, obesity and other traits of the metabolic syndrome [83, 88]. Indeed, lipase inhibition was shown to reduce postprandial triglycerides and fasting LDL, to accelerate weight reduction, to improve metabolic control in type 2 diabetes, and to prevent type 2 diabetes [17, 29].

An inhibition of the lipase can be achieved through a direct interaction with either the lipase or colipase or through an inhibition of enzyme adsorption by interaction with the oil–water-interface.

Protamine is a naturally occurring protein with a basic character obtained from the sperm of various fish species. In an animal trial, protamine decreased postprandial triglyceride levels [99]. In a human trial, we did not see an effect when we compared 0.1, 0.5 and 2.5 g protamine from salmon given in a liquid mixed meal with a control group [73]. This, however, may be due to the fact that lipase inhibitors are less effective in liquids than in solid meals [10].

Kagawa et al. [36] described a hydrolysate from bovine hemoglobin with lipase inhibitory action. This hydrolysate consists of peptides with mainly 3 or 4 amino acids and with a molecular weight range from 100 up to 1,500 Da. It significantly decreased postprandial triglycerides in humans [35]. The tetrapeptide VVYP was identified as a substance with the strongest lipase inhibitory effect [36].

However, a recently published study in humans could not demonstrate more fecal fat or increased fat oxidation with globin peptides [70]. Proteins isolated from wheat germ (24.4 and 27.5 kDa) [7], wheat flour (25 and 28 kDa) [94], soybean cotyledon (80 kDa) [78] and defatted rice bran showed lipase inhibitory action in vitro or reduced the plasma triglyceride levels in rats [100].

To study the hypolipidemic effect of fish protein a protein hydrolysate of flesh remnants on salmon bone frames (FPH) were fed to genetically obese Zucker (fa/fa) rats and several parameters involved in lipid metabolism were compared to soy protein and casein feeding [103]. FPH reduced mRNA levels of δ-5- and δ-6-desaturase compared to casein. The ratio of HDL cholesterol:total cholesterol was greater in Zucker and in Wistar rats fed with fish protein hydrolysate and soy protein compared to those fed with casein.

Table 4 Lipid metabolism modulating proteins and peptides

Protein/peptide	Effect	Model	Refs
Protamine	pp. Triglycerides ↓	Animal	[99]
	pp. Triglycerides →	Human	[73]
Globin digest	pp. Triglycerides ↓	Animal	[36]
	pp. Triglycerides ↓	Human	[35]
	Fecal fat →	Human	[70]
	Fat oxidation →		
	Weight →		
VVYP	pp. Triglycerides ↓	Animal	[36]
	HTGL ↑		
Proteins from			
Wheat germ	Lipase activity ↓	In vitro	[7]
wheat flour	Lipase activity ↓	in vitro	[94]
Soybean cotyledon	Lipase activity ↓	In vitro	[78]
Defatted rice bran	Lipase activity ↓	In vitro	[100]
Fish protein hydrolysate	mRNA of-desaturases ↓	Animal	[103]
	HDL-C/total C ↑		
	ACAT ↓		
Lupin protein isolate conglutin γ	Total C ↓, VLDL- + LDL-C ↓	Animal	[89]
	LDL-uptake ↑, LDL-degradation ↑	Cell culture	
α' Subunits of soybean 7S globulin	Plasma C ↓, triglycerides ↓	Animal	[20]
	VLDL receptor binding ↑		

pp Postprandial, *C* cholesterol, *HTGL* hepatic triglyceride lipase, *ACAT* acyl-CoA:cholesterol acyltransferase
↑/↓/ → Increase/decrease/no effect

In addition, the acyl-CoA:cholesterol acyltransferase (ACAT) activity was lower in Zucker rats fed with fish protein hydrolysate than with casein. Because higher ACAT activity is associated with the progression of atherosclerosis [6, 9], the authors conclude that FPH has cardioprotective properties.

Lupin protein isolate, when fed to rats (50 mg/day by gavage for 2 weeks) in a casein-based diet containing cholesterol and cholic acid, reduced the plasma concentrations of total and VLDL + LDL cholesterol by 21 and 30%, respectively (both $P < 0.001$) [89]. One purified minor component, conglutin γ, upregulated LDL uptake and degradation in HepG2 cells with a maximal increase of 53 and 21% (both $P < 0.05$), respectively.

Duranti et al. [20] provided evidence for an estimated 35% lowering of plasma cholesterol and plasma triglycerides in rats by feeding α' subunits of soybean 7S globulin in a hypercholesterolaemic diet. Furthermore, the activity of the liver β-VLDL receptors of rats fed the α' subunits of soybean 7S globulin had a 96% increase in binding, compared to a hypercholesterolaemic control diet. Table 4 summarizes the effects of proteins and peptides on lipid metabolism.

Peptides with other bioactive effects

Lactoferrin has been reported to prevent microbial growth, and it has been assumed that this effect is due to its ability to bind iron and to deprivate this

essential mineral from microorganisms [8]. Further studies revealed that lactoferrin's bactericidal activities are a result of a direct interaction between the protein and the membrane of Gram-negative bacteria [21]. Moreover, peptides released from lactoferrin by digestion with pepsin or by heat treatment at an acidic pH showed antimicrobial effects [74, 97]. In addition, peptides generated by enzymatic cleavage from α_{s2}-casein, α-lactalbumin and β-lactoglobulin exhibited antimicrobial activities [64, 111].

Some peptides have the ability to bind to opiate receptors and have opiate-like effects, which can be reversed by an opiate antagonist like naloxone. Wheat gluten, α_s-, β-caseinates and hemoglobin have been shown to serve as sources of food-derived peptides with opioid activity after peptic hydrolysis [63].

Tryptic peptides derived from bovine κ-casein with an effect on platelet coagulation are referred to as casoplatelins. Several casoplatelins have been reported to have an antithrombotic activity in vitro [33] and in guinea pigs after parenteral administration [4]. KRDS, a peptide analogue (f39–42) from human lactoferrin, inhibited ADP-induced platelet aggregation after parenteral administration [32].

Several growth factors occur in bovine milk [16]. The insulin-like growth factors IGF I and IGF II promote cell proliferation and differentiation, the transforming growth factor TGF-α stimulates the proliferation of cells in the connective tissue and inhibits growth of other cell types like lymphocytes and epithelial cells. Whether these properties have implications for human health needs further investigations [86].

Papenburg et al. [62] found whole whey protein to have anticancer activity in A/J mice compared to casein and Purina mouse chow. Either lactoferrin [102] or bovine serum albumin [42], or both, may be responsible for this effect. A α-lactalbumin–oleic acid complex formed in acid condition induces apoptosis in tumor and transformed cells in vitro [92]. The topical application of this complex reduced skin papillomas, resistant to conventional treatment, by 75% whereas the placebo leads to a reduction of only 15% in a double-blind study [24].

Two antioxidative enzymes derived from milk, superoxide dismutase and catalase, prevent the formation of radicals, scavenge radicals or hydrogen peroxide and other peroxides in vitro [43]. Lactoferrin [87], peptides obtained from casein [68, 69] and soybean protein [11] yielded antioxidative activity through inhibiting enzymatic and non-enzymatic lipid peroxidation in vitro. These antioxidative substances, however, were all tested in vitro only and have to be tested for anti-atherogenity activity in animal and human trials.

Takahashi [93] has reported on a peptide, isolated from the tryptic digestion of a soluble protein of rice, which showed ileum contracting features.

Trompette et al. [98] demonstrated an increased protection of the gut through a stimulation of intestinal mucus release after the in vitro administration of β-casomorphin-7 to isolated perfused rat jejunum.

CPPs have been shown to have strong anticariogenic effects [67]. Apart from their ability to bind Ca^{2+} ions, CCPs may also improve the solubility and availability of other minerals like zink, magnesium, iron, chromium and selenium. Thus, CCPs may reduce the risk of a deficiency in these essential minerals [82].

Potential application in functional food or medical products

There certainly is a potential for the application of bioactive proteins and peptides as functional foods or pharmaceutical products. Health-conscious and people with specific demands or opportunities may be target groups for foods enriched with antihypertensive, antilipemic and antioxidative proteins and peptides, substances, that stimulate mineral absorption and immunomodulatory proteins and peptides. The immunosuppressive properties of some milk components may be used to treat people with intestinal inflammation.

The most widely used method for yielding bioactive peptides in vitro is the enzymatic hydrolysis by pancreatic enzymes, especially trypsin. Once the amino acid sequence of the molecule is known, the peptide can be synthesized by chemical synthesis or recombinant DNA technology. Chemical synthesis is very useful for the production of short sequences, the recombinant DNA technology is very useful for the generation of larger peptides.

Another possibility for generating bioactive substances with peptide character may be achieved through the development of stable bovine colostrum concentrates or through gaining milk from hyperimmunized dairy cows.

Conclusion

There are plenty of reports on the bioactivity of proteins and peptides in vitro. Such data, however, are insufficient in claiming an effect on human health since the active compound may be degraded during digestion, may not be absorbed or not attain the appropriate concentrations in blood and target tissues that are required for acting significantly. Certain processing procedures, especially heating, may influence the bioactivity and may also lead to the formation of undesired toxic, allergic or carcinogenic substances. In addition, the bioactivity may be re-

duced through molecular alteration during food processing or interaction with other food ingredients. The bitter taste of protein hydrolysates prevents the use of many products as food additives. The challenge for food technologists will be to develop functional foods and nutraceuticals without the undesired side effects of the added peptides, and to retain the stability of the added peptides within the shelf life of the product. Therefore, controlled trials in humans are mandatory when claiming a health effect for a food. So far, evidence for such health effects exists only for a few proteins and peptides. The sour milk products Calpis™ and Evolus®, containing antihypertensive tripeptides, are examples for products available on the market whose effects on blood pressure have been scientifically proven in human trials [28, 85]. Further effects of bioactive peptides and proteins have been demonstrated in human trials: a decrease in postpandrial triglycerides after the administration of lipase-inhibitory globin digest [35], an increase in the percentage of granulocytes of the total leukocytes and monocytes after supplementation with lactoferrin [71], and an increase in bone mineral density and an improvement of bone metabolism in menopausal [3] and young women [95] after supplementation with milk basic protein.

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